**Background**

We developed a novel qPCR-based pre-amplification (PreAmp) technology that can increase the abundance of over 350 target genes in a single step. To assess bias introduced by PreAmp, we utilized SsoAdvanced PreAmp reagents, which allow for high multiplex and high amplification conditions. We do detect dynamic range bias if a target gene is highly abundant and PreAmp occurred for 16 or more PCR cycles; however, this type of bias is rarely needed. To evaluate PreAmp performance in a gene expression profiling experiment, we analyzed a panel of genes that are regulated differently during neural differentiation of NTERA2 cells. We found that PreAmp maintains patterns of gene expression across samples, the same biological insights would be derived from PreAmp-represented and a standard gene expression profiling experiment. Our PreAmp technology thus facilitates accurate analysis of relatively limited samples in gene expression profiling experiments.

**Model systems utilized**

NTERA2 cells (NT2) are a model system of human stem cell biology. When treated with retinoic acid (RA), NT2 differentiate into neurons. During differentiation, the expression of stem cell transcription factors decreases and the expression of neural transcription factors increases. We analyzed RNA isolated from NT2 cells treated with RA for 0-7 days and quantified along with the natural RNAs. The amount of each ERCC RNA in a mixture is precisely defined; the performance of an RNA quantification workflow is determined by comparing the measured amount with the actual, defined amount of each ERCC control RNA (1).

**SsoAdvanced PreAmp performance**

SsoAdvanced PreAmp has an input dynamic range of 10 pg to 1 ng cDNA

PreAmp was performed on 10 pg to 1 ng cDNA, in triplicate target genes with similar expression levels were analyzed by qPCR. The results show that the performance of SsoAdvanced PreAmp is similar to the performance of qPCR using not-pre-amplified samples. The PreAmp has a high capacity to amplify target genes even if there is a 3-log difference in input gene expression levels (e.g., a ratio of 10,000,000:1).

SsoAdvanced PreAmp is efficient for up to 20 PreAmp cycles

PreAmp was performed on 10 pg to 1 ng cDNA for 10,000 PreAmp cycles. qPCR analyses show changes in 10,000 pre-amplified samples increased by 2 to 10,000-fold over not-pre-amplified samples. The final amplified product can quantitate the entire dynamic range of the ERCC controls. Pre-amplification for up to 16 PCR cycles can also accurately quantify the entire ERCC assay set and, therefore, can be used in most PreAmp experiments. The fold-change results are consistent with those obtained from standard qPCR experiments.

**References**